ESTIMATING MOIST-SOIL SEEDS AVAILABLE TO WATERFOWL WITH DOUBLE SAMPLING FOR STRATIFICATION

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Moist-soil managers manipulate hydrology, soils, and vegetation to provide habitat and foods for waterfowl and other wildlife in seasonally flooded herbaceous wetlands. Increasing seed availability for waterfowl is a priority, but managers also provide resources such as invertebrates, tubers, and browse (Fredrickson and Taylor 1982). An important principle in moist-soil management is maintaining a large component of early-successional plant species whose reproductive strategies include production of abundant seed (Cronk and Fennessy 2001). Low and Bellrose (1944) first referred to the annual species that colonize mudflats as moist-soil plants and documented their potential seed production. Fredrickson and Taylor (1982) developed guidelines for modern moistsoil management in the 1970s and use of moist-soil methods increased rapidly thereafter (Fredrickson 1996). In the Mississippi Alluvial Valley (MAV), state and federal wildlife agencies now manage >8,000 ha in 300 impoundments for moist-soil habitat (U.S. Fish and Wildlife Service 2002).

Several methods have been used to quantify seed availability in moist-soil habitats. Harvesting seeds from inflorescences has been the most common method of estimating seed production of individual plant species (Low and Bellrose 1944, Fredrickson and Taylor 1982, Haukos and Smith 1993). Other researchers have tried to simplify estimating seed production by developing species-specific predictive models relating seed yield to plant morphology (Laubhan and Fredrickson 1992; Gray et al. 1999 *a,b*). We believe more effort is needed to develop methods to estimate seed availability for management units rather than for individual species because of the increasing number of impoundments managed and the need to under-

stand the role of moist-soil habitat in meeting food requirements of nonbreeding waterfowl (Reinecke and Loesch 1996, Miller and Newton 1999).

Double sampling for stratification (hereafter double sampling; Thompson 1992:143) potentially increases precision of estimates but does not assume that the stratum membership of plots or the sizes of strata are known. Sample units (plots) are assigned to strata during the first sampling period based on predetermined criteria, and stratum sizes are estimated as proportions of plots assigned to strata in the first sample. Successful stratification reduces sampling costs by decreasing the size of the second sample needed to achieve the desired level of precision to inform management decisions. We used double sampling to estimate seed availability in moist-soil impoundments. Our strategy involved 2 sampling steps. We selected a large first sample of plots and used qualitative criteria that we believed were correlated to seed availability to assign plots to different strata (levels of seed availability). Then we selected a second (sub)sample of the first sample, and in these plots we measured seed availability by collecting soil cores and plant inflorescences just before waterfowl arrived. We used double sampling to achieve the increased precision associated with stratified designs, and we measured seed availability by collecting soil cores and inflorescences just before waterfowl arrived to assess the abundance of resources actually available to the birds. Previous studies (Low and Bellrose 1944, Laubhan and Fredrickson 1992, Haukos and Smith 1993) have assumed that no mortality of seeds occurs between the time seeds are harvested by researchers during the growing season and the time waterfowl arrive in fall or winter.

Our general objective was to determine if double sampling would provide precise, cost-effective, and unbiased estimates of seed availability in

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moist-soil impoundments. Specific objectives were to (1) estimate mean seed availability for 3 impoundments in each of 2 years; (2) compare the statistical and cost efficiency of double sampling to that of simple random sampling; and (3) determine if incomplete seed recovery from soil cores leads to biased estimates of seed availability.

Study Area

We conducted our study during autumns 2001 and 2002 at the 5,284-ha Yazoo National Wildlife Refuge (YNWR) located 48 km south of Greenville in west-central Mississippi, USA. We collected data from impoundments in the Cox Ponds wetland complex (hereafter Cox Ponds). The Cox Ponds impoundments (n=14, $\bar{x}=5.9$ ha, range = 2.8–8.7 ha) were managed as an integrated complex following principles in Fredrickson and Taylor (1982). Each year, 3–5 of the 14 impoundments were managed as mudflats for shorebirds, permanent wetlands for wading birds, and moist-soil vegetation for waterfowl.

Methods

Sampling Design—Because management treatments rotated annually, we had 3 moist-soil impoundments available to sample each year and had to sample impoundment 8 in both years. We obtained digital vector data (U.S. Fish and Wildlife Service 2002) representing boundaries of the impoundments and used ArcView® GIS 3.2a (Environmental Systems Research Institute 1996) to select a systematic first sample (n = 340-381) of $1-m^2$ plots (0.4–1.2% of the total area) in each impoundment. In mid-September, we used a differential global positioning receiver to locate plots, assess expected seed availability, and assign plots to 1 of 2 or 3 strata within impoundments. The number of strata selected and criteria for assigning plots to strata were somewhat arbitrary. The objective in double sampling is to create strata whose means differ within impoundments and the sum of whose variances is less than that for a simple random sample. Our sample designs included 2 strata (low vs. high expected seed availability) in 1 impoundment (#8, which was sampled both years) and 3 strata (low vs. medium vs. high) in the other 4 impoundments. We used 2 primary criteria to assess expected seed availability: (1) presence and potential seed production of known plant species (cf., Fredrickson and Taylor 1982), and (2) relative abundance of seed visible on the soil surface. We developed criteria for the strata independently in each impoundment and

did not expect low, medium, and high density strata in different impoundments to have the same mean seed availability.

For each impoundment, we estimated stratum sizes as proportions using data from the first samples (PROC SURVEYMEANS; SAS Institute 1999). Then, we used PROC SURVEYSELECT to draw a second (sub)sample (m = 35 plots) from each first sample. In each impoundment, the proportion of the 35 plots selected from each stratum reflected the estimated size of that stratum.

Measurement of Seed Availability—During mid-October, we went to all 35 second-sample plots in each impoundment, clipped inflorescences within a 0.25-m² frame, and collected soil cores with a depth and diameter of 10 cm. We soaked soil cores in a 3% solution (1:32) of hydrogen peroxide (H₂O₂) for 3–5 hrs to disperse clays (Bohm 1979:117) and conducted a test to ensure the oxidizing agent H₂O₂ had no effect on the mass of barnyard grass (Echinochloa crusgalli) seeds (K. J. Reinecke and K. M. Hartke, unpublished data).

We washed samples with water over a set of 2 or 3 sieves, depending on the amount and coarseness of plant detritus. The set included a No. 5 (4 mm) or No. 10 (2 mm) sieve combined with a No. 45 (355) μm) sieve. After removing seeds from the coarse sieve(s), we dried material remaining in the No. 45 sieve. We then used a second set of 3 sieves to separate large (retained by No. 35 [500 µm] or No. 20 [850 μm] sieves) and small seeds (retained by No. 45 sieve). We removed large seeds from the first 2 sieves and determined mass (to the nearest 0.1 mg) after drying for 48 hrs at 50°C. Then, we distributed material retained by the No. 45 sieve uniformly over a numbered grid of 100 equal sized cells and drew a random subsample of 25. We used a binocular microscope to remove small seeds from the selected cells. After determining dry mass of small seeds in the subsample, we multiplied by 4 to estimate the mass of small seeds in soil cores. We calculated total mass of seeds in soil cores as the sum of the masses of large and small seeds. After airdrying plant inflorescences, we held them over the 3 sieves used to separate large and small seeds, and threshed out the seeds they contained. After drying and weighing seeds from inflorescences, we added the mass of seeds in soil cores and the mass of seeds in inflorescences to create a response variable (in kg/ha) for estimating mean seed availability.

Assessment of Recovery of Seeds from Soil Cores—We quantified the percentage of barnyard grass seeds recovered from soil cores containing a range of seed densities to determine if incomplete recov-

ery biased estimates of seed availability. We used barnyard grass in this experiment because seeds of this species are large and most seeds (83% of total mass) in soil cores were large. We prepared test cores by adding known numbers of seeds to soil (n = 12 cores; 2 cores each with 0, 12.5 [12 or 13], 25, 50, 100, and 200 seeds) in quantities we were likely to encounter in field samples (equivalent to 0-750 kg/ha). We prepared test cores with a silty-clay soil and added representative amounts of organic matter and small seeds (Leptochloa fascicularis) to increase realism. We interspersed test cores with actual cores obtained in the field study to ensure similar processing. We weighed any detritus that remained after processing to determine if its mass influenced seed recovery.

Analyses—After obtaining simple means (PROC MEANS; SAS Institute 1999) for seed availability within strata, we calculated means (\bar{x}_{DS}) and variances ($v[\bar{x}_{DS}]$) for impoundments using estimators appropriate for double sampling (Lohr 1999:384–385):

$$\overline{x}_{DS} = \sum_{h=1}^{II} \frac{n_h}{n} \ \overline{x}_h,$$

$$v(\overline{x}_{DS}) = \sum_{h=1}^{H} \frac{n_h - 1}{n - 1} \frac{n_h}{n} \frac{s_h^2}{n} + \frac{1}{n - 1} \sum_{h=1}^{H} \frac{n_h}{n} (\overline{x}_h - \overline{x}_{DS})^2,$$

where h represented the strata, n_h was the number of plots among n in the first sample assigned to stratum h, and m_h , \bar{x}_h , and s_h^2 were the sample sizes, means, and variances for the second samples in stratum h, respectively.

We calculated design effects and effective sample sizes (Lohr 1999:239-242) to assess the efficiency of double sampling. Design effects are ratios of the variance of a statistic obtained using a complex sample design to the variance of the same statistic calculated from a simple random sample. To estimate design effects for each impoundment, we used the variances from double sampling described above and obtained variances of means for simple random samples from PROC MEANS. A design effect of 1.0 indicates that 2 sampling methods provide equivalent statistical precision but not necessarily at the same cost. We divided the sample size used in double sampling (m = 35) by the design effects to estimate effective sample sizes—the sizes of simple random samples that would provide equal precision.

We used regression analysis (PROC GLM; SAS Institute 1999) to determine whether percentages of barnyard grass seeds recovered from test soil cores varied with the number of seeds initially present in those cores, the dry mass of detritus, or the interaction between these 2 variables. We used the ESTIMATE statement of PROC GLM with the best model to estimate the percentage of seeds recovered and the ratio between seeds added and recovered, which represented the degree of potential bias.

Results

Mean seed availability varied from 331-1,084 kg/ha among impoundments and between years (Table 1). The unweighted mean of impoundment means was 603 kg/ha. Within impoundments, mean seed availability in 3 sampling strata with high expected seed density was 1,037–1,562 kg/ha, but no high density stratum occupied >50% of an impoundment (Table 1). With 1 exception, barnyard grass and smartweeds (Polygonum pensylvanicum, P. lapathifolium, P. densiflorum) dominated all strata with mean seed availability of ≥711 kg/ha (K. J. Reinecke and K. M. Hartke, unpublished data). The exception occurred in the low density stratum of impoundment 4 (Table 1), where mud-plantain (Heteranthera reniformis) produced an unexpected abundance of small seeds. Over impoundments and years, large seeds contributed most (83%) of the total mass of available seeds. Most (93%) of the total seed mass was recovered from soil cores rather than from inflorescences.

Precision of impoundment means, expressed as coefficients of variation (CV), ranged from 7.0 to 11.5%, although most were <10% (Table 2). Design effects for double sampling ranged from 0.44 to 1.02 (Table 2). Effective sample size was approximately 70 for 3 impoundments (2, 6, 9) but near 35 for impoundments 8 (both years) and 4. By increasing effective sample size to 70 in impoundments 2, 6, and 9, double sampling provided benefits equal to the costs of collecting (approx 3 days) and processing (approx 15 days) 35 additional samples.

The soils we used to prepare test samples apparently contained few, if any, barnyard grass seeds because we did not recover any seeds from test cores where none were added. Overall, we recovered 86.7% (672/775) of barnyard grass seeds added to test cores. Percentages of seeds recovered from test cores did not vary with the number of seeds added ($F_{1,6} = 0.57$, P = 0.477), amount of detritus ($F_{1,6} = 0.00$, P = 0.973), or the interaction between these 2 factors ($F_{1,6} = 0.39$, P = 0.558). Using the null model, the estimated percentage of seeds recovered was $89.5\% \pm 2.2$ (SE), and the

Table 1. Mean mass (kg/ha) of moist-soil seeds available to waterfowl in 6 impoundments (1 sampled in both years) at Cox Ponds wetland complex, Yazoo National Wildlife Refuge, Mississippi, USA, Oct 2001 and 2002. Impoundment means were estimated using double sampling for stratification (Lohr 1999:385).

Year	Impoundment	Stratum	Sizea	n ^b	n_h^c	m^{d}	m_h^{d}	\bar{x}	SE	LCLe	UCLe
2001	4			340		35		799	73	653	945
		Low	0.46		156		16	728	147	415	1,041
		Medium	0.30		103		10	720	63	577	862
		High	0.24		81		9	1,037	87	837	1,236
	8			354		35		331	38	254	408
		Low	0.72		256		25	341	46	246	435
		High	0.28		98		10	305	71	145	465
	9			353		35		348	34	280	416
		Low	0.52		185		18	173	19	133	212
		Medium	0.29		102		10	432	68	279	584
		High	0.19		66		7	711	127	400	1,023
2002	2			372		35		1,084	76	931	1,236
		Low	0.31		117		11	320	82	138	502
		Medium	0.21		78		8	1,145	116	871	1,420
		High	0.48		177		16	1,562	130	1,286	1,831
	6			377		35		640	54	532	748
		Low	0.54		202		18	352	66	213	490
		Medium	0.22		83		8	827	44	722	931
		High	0.24		92		9	1,103	149	759	1,447
	8			381		35		415	38	340	490
		Low	0.65		248		22	358	47	259	456
		High	0.35		133		13	523	61	391	655

ratio between seeds added and recovered (potential bias correction) was 1.123 ± 0.027 .

Discussion

Controlling sample size is critical in estimating seed availability because data collection requires costly field and laboratory procedures. Double sampling is efficient when (1) a response variable is heterogeneous; (2) variables correlated to the response variable can be used in the first sample to assign plots to strata with different means; (3) first samples are large enough that estimation of stratum sizes contributes little to the overall variance; and (4) the value of increased precision resulting from stratification exceeds the cost of collecting the first sample. In our study, impoundments 9 (2001) and 2 and 6 (2002) satisfied all criteria for effective double sampling. Seed availability in these impoundments was highly variable and stratum means separated predictably (Table 1). Effective sample sizes were twice actual sample sizes (Table 2), and double sampling provided benefits equivalent to 15-20 days of additional work. Double sampling failed to increase effective sample size in impoundment 4 (2001) because we violated criterion (2). We did not anticipate abundant production of small seeds by mud-plantain and assigned plots dominated by this species to the low density stratum, thereby increasing the stratum variance and causing means of the low and medium density strata to overlap (Table 1).

Based on preliminary observations, we suspected impoundment 8 would not satisfy criterion (1) either year; nevertheless, we applied double sampling to assess our ability to discriminate small differences in seed availability within the impoundments. As expected, mean seed availability in impoundment 8 was low in both strata and years (≤523 kg/ha; Table 1), and variation was insufficient to create effective strata. Double sampling and simple random sampling had similar effective sample sizes in impoundment 8 (Table 2), but double sampling required 3 additional days to assign plots to strata in the first sample.

Overall, when appropriate criteria were met, double sampling provided estimates of a given precision with samples half as large as those required in a simple random sampling design. However, doubling sampling was sensitive to accurate stratification of plots in the first sample

^a Stratum size as a proportion = n_h / n . ^b Sample size for the first phase of double sampling.

 $^{^{\}mathrm{c}}$ Number of plots among n in the first sample assigned to stratum h.

d Sample size for the second phase of double sampling; the second sample was a random (sub)sample of the first sample and allocated among strata proportional to size.

e 95% confidence limits: lower (LCL) and upper (UCL).

Table 2. Coefficients of variation (CV; %), design effects (deff), and effective sample sizes (eff_n) for a double sampling design used to estimate the mean (\bar{x}) availability of moist-soil seeds (kg/ha) in 6 impoundments (1 sampled in both years) at Cox Ponds wetland complex, Yazoo National Wildlife Refuge, Mississippi, USA, Oct 2001 and 2002.

Year	Impoundment	m ^a	\bar{x}	SE	CV	$v(\bar{x}_{DS})^{b}$	$v(\bar{x}_{SRS})^c$	deff ^d	eff _n e
2001	4	35	799	73	9.1	5,356	5,653	0.95	37
	8	35	331	38	11.5	1,476	1,443	1.02	34
	9	35	348	34	9.8	1,155	2,307	0.50	70
2002	2	35	1,084	76	7.0	5,815	13,139	0.44	79
	6	35	640	54	8.4	2,919	5,658	0.52	68
	8	35	415	38	9.2	1,411	1,542	0.92	38

a Sample size for measuring the primary variable in the second phase of double sampling.

and unnecessary in situations where the response variable was relatively homogeneous.

Because most seeds (93% of total mass) were in soil cores rather than inflorescences when we sampled, the primary bias potentially affecting our measurements was incomplete recovery of seeds from cores. In the blind experiment we conducted to assess this bias, the estimated proportion of seeds recovered from test cores (89.5% \pm 2.2) and the bias correction (1.123 \pm 0.027) indicated we underestimated true seed availability by approximately 12%. Little work has been done to assess potential biases associated with other methods of measuring seed availability. Seed harvesting has been used to obtain most estimates of seed availability (Low and Bellrose 1944, Fredrickson and Taylor 1982, Haukos and Smith 1993, Gray et al. 1999c). Harvesting allows accurate measurement of seed availability for waterfowl for plant species whose seeds mature and are harvested when waterfowl arrive, but many species have seeds that mature earlier in the growing season. We believe researchers should investigate the possibility that significant seed mortality occurs between the time seeds are harvested and waterfowl use impoundments, and assess how this may bias estimates of seed availability.

Deciding how to select plots and measure the variables of interest in plots that are selected comprises a sampling strategy that determines the accuracy (i.e., precision + bias²) of estimates (Thompson 1992). We selected plots with double sampling, measured plots by collecting soil cores and plant inflorescences just before waterfowl arrived, and determined the extent of bias in our measurements. Thus, we believe our sampling strategy satisfied Anderson's (2001) recommendation that researchers use more explicit sample designs and evaluate sources of bias in measurements.

Conservation strategies of the Lower Mississippi Valley Joint Venture (LMVJV; Lower Mississippi Valley Joint Venture Management Board 1990) advocate increasing food resources to achieve waterfowl population goals in the MAV (Loesch et al. 1994, Reinecke and Loesch 1996). Moist-soil management is an important component of the conservation strategy because >8,000 ha in 300 impoundments are managed as moist-soil wetlands (U.S. Fish and Wildlife Service 2002) and waterfowl food resources are decreasing on private agricultural land (Manley et al. 2004, Stafford 2004). In the past, there has been considerable uncertainty about food abundance in moist-soil habitats. Fredrickson and Taylor (1982) reported that seed availability was 1,629 kg/ha in Missouri where intensive management was practiced. In contrast, Reinecke et al. (1989) recommended the LMVJV use a conservative estimate of 450 kg/ha in regional planning decisions because few impoundments in the MAV were managed intensively. In our study, estimates of seed availability from YNWR varied among 6 impoundments (1 sampled in 2 years) from 331–1,084 kg/ha (Table 1) with a mean of 603 kg/ha. Moser et al. (1990) reported seed availability in Arkansas varied in 3 impoundments over 3 years from 253–1,288 kg/ha with a mean of 613 kg/ha, and Penny (2003) recently reported mean seed availability was 611 \pm 146 kg/ha for a sample of 26 impoundments in the MAV. Apparently, seed availability in certain impoundments or plant stands can reach the level attributed to intensive management (1,629 kg/ha; Fredrickson and Taylor 1982), but estimates of seed availability for entire impoundments or multiple impoundments are rarely this high. Further reducing uncertainty about food abundance in moist-soil

 $^{^{\}mathrm{b}}$ $v(ar{x}_{D\mathrm{S}})$ is the variance of mean seed mass obtained with double sampling.

 $^{{}^{\}rm c}$ $v(\bar{x}_{SRS})$ is the variance of mean seed mass obtained with a simple random sample.

d Design effect (*deff*) is the ratio of $v(\bar{x}_{DS})$ to $v(\bar{x}_{SRS})$.

^e Effective sample size (eff_n) is the sample size used for double sampling (m) divided by the design effect (deff), and it represents the size of a simple random sample that would provide precision equal to that of the double sample.

impoundments in the MAV will require the kind of large-scale sampling done recently by Stafford (2004) to determine food availability for waterfowl in ricefields throughout the MAV.

Management Implications.—Double sampling can be an effective strategy for increasing precision or decreasing costs of estimating moist-soil seeds available to waterfowl over entire management units. Efficiency of double sampling increases with the extent to which seed availability varies. Double sampling has potential as a strategy for increasing precision in measuring responses when experimental treatments (e.g., irrigation, tillage) are applied to impoundments to increase seed availability. Estimates of mean seed availability over entire impoundments at YNWR ranged from 331 to 1,084 kg/ha and exceeded 1,200 kg/ha only in a limited portion (48%) of 1 impoundment. Our results highlight the need to obtain additional data from impoundments throughout the MAV to reduce uncertainty about the extent to which food abundance in moist-soil impoundments contributes to regional objectives for managing waterfowl foraging habitat.

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